Environ. Biosafety Res. 1 (2003) –

Session on: Transgenic insects for pest management programs: status and prospects

Marjorie A. HOY, Chairperson

Dept. of Entomology & Nematology, University of Florida, P.O. Box 110620, Gainesville, FL 32611-0620, USA

SPEAKERS

Analysis of risks of transgenic insects for pest management: past and future guidelines
Marjorie A. Hoy
University of Florida, USA

Transformation of mosquito vectors of disease: goals and risk analyses
Chris Curtis
London School of Hygiene and Tropical Medicine, UK

Transgenic pink bollworms: evaluation of risks of releases in genetic control projects
John Peloquin
University of California Riverside, USA

Chagas disease vectors that do not transmit the disease agent
Ravi Durvasula
Yale University School of Medidine, USA

Transgenic Mediterranean fruit flies for sterile insect release programs
Alan Robinson
FAO/IAEA Agriculture and Biotechnology Laboratory, Austria

E-mail: mahoy@mail.gnv.ifas.ufl.edu

This session was the first to cover the topic of transgenic insects in any of the International Symposia on the Biosafety of Genetically Modified Organisms. The objectives were to provide an overview of the goals of various programs around the world that would involve the use of transgenic insects or other arthropods in applied pest management programs. The five speakers were asked to review the goals of programs and the risk issues that might be associated with specific transgenic insects. Although the session did not include speakers representing all the programs in progress, it provided an overview of the diversity of goals, methods, and progress towards implementation.

Genetic modification using recombinant DNA methods can now be used, almost routinely, to transform pest and beneficial insects. Goals include modifying mosquitoes, and other insects that transmit human and animal diseases, so that they are unable to transmit the causal pathogens. Recombinant DNA methods could improve genetic control programs by producing sterile male insects or producing only females. Other goals include producing honeybees that are disease resistant and silk moths that produce new types of silk. Some insects are being transformed to produce drugs and vaccines. Natural enemies used in biological control programs could be modified to enhance their effectiveness in several ways.

The session began with an overview by M. Hoy, in which she noted that risk assessments in the USA must be conducted prior to releasing transgenic insects into the environment for short term experiments. No guidelines are available for risk assessments of transgenic insects prior to their permanent establishment in the environment, which is a goal of some genetic manipulation programs.

Potential risk issues to be resolved prior to releases include whether: the inserted gene(s) (trait) is stable; the traits (especially pesticide or antibiotic resistance genes) can be horizontally transferred to other populations or species; released insects will perform as expected with regard to their geographic distribution, host or prey specificity and other biological attributes; released insects will have unintended environmental effects; and, in the case of short-term releases, the released insects can be recovered from field sites. Risk assessments of fitness and host specificity are relatively easy to assess in the laboratory, but the potential risks of horizontal gene transfer and unintended effects on ecosystem function are much more challenging.

In the USA, permission to release a transgenic insect will have to be obtained from (several) regulatory agencies. Two releases of transgenic arthropods have occurred in the USA; one of a predatory mite (Acarina) that contained a *lac*Z marker gene and one of a pink bollworm moth that contained a green fluorescent protein gene. Releases were made into small plots and were intended to be short term experiments. Current regulations of the U.S. Department of Agriculture require the researcher to retrieve all transgenic arthropods from the environment at the end of the experiment.

If transgenic arthropod strain(s) perform well and risk assessments are completed satisfactorily, permanent releases into the environment may be allowed, but U.S. guidelines for such releases are lacking. Many pest management programs, especially those involving *replacement* of pest populations by a transgenic population, will require permanent establishment of the genetically modified strain (or of the transgene in the wild population) in the environment. Several drive mechanisms, including the release of active transposable elements or symbionts such as *Wolbachia*, have been proposed to insert genes into populations, but analyses of the potential risks of such drive mechanisms have not been carried out.

M. Hoy concluded with a plea that international guidelines be developed for risk analyses of transgenic arthropods because most are highly mobile and could move beyond individual countries' boundaries. Such guidelines would provide an impetus to the deployment of transgenic arthropods in pest management programs.

The second speaker was C. Curtis, who presented a talk on "Possible ways of using transgenic mosquitoes for malaria or dengue control and risk assessment". The problems of insecticide resistance in some mosquito populations and the difficulties of implementing traditional control methods justify the development of improved control methods, including, potentially, the use of transgenic insects to eradicate wild populations of these vectors of disease. The use of transgenesis to improve sterile insect release programs might be achieved by improving methods for separating the sexes in mass rearing factories so that males only are produced. One such method, called RIDL, might involve using a dominant lethal gene associated with a female-specific promoter so that expression of the dominant lethal is switched off so long as a particular nutrient is provided to the breeding stock; when, however, insects are being reared for release the nutrient could be removed, causing the death of all females. This would reduce costs of producing the millions of sterile males needed for control programs and reduce the likelihood of releasing females that could bite or transmit disease. Risk issues associated

with the RIDL method include the need to: ensure that only males are released; eliminate any revertants in the breeding stock; evaluate the risk of horizontal gene transfer of the transgene(s); communicate clearly with the human population about the goals of the proposed control program.

The development of *Plasmodium*-refractory strains (strains that cannot transmit malaria) by transgenesis also was reviewed, and the difficulty of ensuring that the refractory trait is tightly linked with the "drive" mechanism used to insert the trait into the wild population was noted as a risk issue. Other potential risks associated with this control approach include the possibility of resistance to the refractory gene developing in *Plasmodium*, which would result in program failure. Another issue of concern was the importance of testing the transgenic mosquitoes to confirm that they are not susceptible to (or vectors of) pathogens other than *Plasmodium* as a result of undergoing genetic modification. Curtis concluded that the best chance of using transgenic mosquitoes may be "against urban vector populations surrounded by a different species in rural areas", because the logistics of deploying a complex genetic control program throughout the vast areas of Africa are daunting.

The third speaker was J. Peloquin, who gave the presentation "Field trials, the permitting process, comments and risks", and provided an overview of a project involving a transgenic pink bollworm, which was genetically modified using the transposable element *piggybac* to contain a modified version of the green fluorescent protein gene as a marker. The pink bollworm, *Pectinopora gossypiella*, is an important pest of cotton and has been the target of a sterile insect release program in California for some time. The initial objective of the program is to develop pink bollworms that can be identified readily by program managers in the sterile insect release program.

The field test of the bollworm strain with a marker gene required a lengthy risk assessment. The transgenic strain containing a green fluorescent gene construct was developed in March 1998; permission to move the transgenic strain from California to Arizona was given in March 1999 by the US Department of Agriculture-APHIS. A draft application to release was submitted in January 2000 to freely release (not into cages) the transgenic pink bollworms into the field site. This application to release was reviewed by the Arizona State Department of Agriculture and the US Department of Agriculture and comments by the public followed which resulted in a modified protocol in which the transgenic bollworms were released into cages.

Releases of the transgenic pink bollworms began October 5–16, 2001 and additional releases occurred during the summer of 2002. Releases were performed in a 3-acre cotton field in Arizona into cages within a plot surrounded by chain-link fencing to limit access by humans and animals, and the site was guarded to prevent vandalism. Safeguards to prevent the accidental escape of the transgenic insects from the cages included: pheromone traps were placed at the edges of the field to capture escaped insects; the field was treated with sterile pink bollworms three times a week; wings of the transgenic females were clipped to prevent them from flying away should they escape the cages; females were restrained during the experiment in "mating stations"; at the end of the experiment, cotton bolls from the release field were destroyed; only irradiated transgenic males were released. The goals of the field trial were to: compare the transgenic and nontransgenic males' responses to pheromones in the field; compare the longevity of the two types of males in the field, and compare the ability of the two types of females to solicit and mate with the two types of males in the field.

One concern raised by reviewers of the proposed releases was the possibility of horizontal gene transfer. J. Peloquin also discussed various statistical and experimental methods for analyzing this, expectedly, rare event.

A. Robinson presented a talk on "Transgenic mediterranean fruit flies for the sterile insect technique". The Sterile Insect Technique (SIT) is used to suppress, eradicate or prevent the establishment of Mediterranean fruit fly (Medfly) populations. Currently, recombinant DNA methods could be expected to improve three aspects of this effective pest management tactic: (1) produce improved male-only strains for release; (2) introduce a phenotypic marker into the Medfly to replace the use of fluorescent dye for marking released insects (which is an important operational aspect of monitoring the progress of an SIT program); (3) develop transgenic strains that exhibit a dominant lethality in the field after release so that irradiation is no longer required to induce sterility in the released males (the most distant goal).

If transgenic Medflies are released that have been sterilized in a traditional manner by irradiation, they cannot become established in the environment and the primary risk might be the horizontal transfer of the transgene to other organisms. Because of concerns about horizontal gene transfer, the use of antibiotic or cell death genes should be avoided in developing transgenic Medflies. If, however, fertile transgenic males are

released that transmit genes to induce death in the embryos of wild females fertilized by these males, an alteration in the lethal system in the released Medflies could allow transgenes to leak into wild populations. Studies will have to be conducted to evaluate transgene stability and how horizontal transmission could be minimized. Research is needed to understand epigenetic interactions that might result in unintended or unexpected transgene activity or repression. New gene transfer systems are required that would allow targeted insertion to reduce the problems of random insertion.

The final speaker was R. Durvasula, who discussed "A paratransgenic strategy for control of chagas disease." Paratransgenesis is the genetic manipulation of commensal or symbiotic bacteria that reside within arthropod hosts. The bacteria can be transformed to produce molecules that interrupt transmission of a target pathogen of humans, other animals, or plants.

The example provided was that of *Rhodnius prolixus*, a reduviid bug vector of Chagas disease, which is caused by *Trypanosoma cruzi*. This disease kills over 50 000 people annually and nearly 90 million are at risk for the disease in Central America and parts of South America. At present, neither a cure nor a vaccine exists for Chagas disease. The transformation of an actinomycete gut symbiont, *Rhodococcus rhodnii*, with a cecropin A gene can result in the death of the infective stages of *T. cruzi* because *R. rhodnii* is an extracellular, intraluminal symbiont in the hindgut of the bug, which is where the infective stage of the pathogen occurs. Other goals of the program include developing engineered antibodies that could be produced by the gut symbiont. Delivery and spread of the transgenic symbiont among natural populations of the bug could be achieved by spreading a simulated fecal paste containing the symbiont, called Cruzigard, in the environment where the bugs hide. If the bugs feed on Cruzigard, they may retain the engineered gut bacteria, which would then produce the antibiotic or antibody, reducing or eliminating the ability of the bug to transmit the trypanosome.

Potential environmental implications of this disease-control strategy include the potential toxicity of the Cruzigard bacteria to humans. Stability of the transgenes and horizontal gene transfer need to be assessed; the likelihood and effect of gene transfer to nontarget bacteria or to nontarget arthropods needs will be assessed.

In concluding remarks, M. Hoy stated that "The insects are coming", meaning that biosafety research on transgenic arthropods should become an important component of this International Symposium in future years. It is essential that regulatory issues be resolved and planning for appropriate risk assessments be initiated so that programs for managing serious arthropod pests with biotechnological methods are not delayed unnecessarily. Furthermore, international risk assessment guidelines should be developed for transgenic insects. Because insects are highly mobile, they do not recognize international borders and could readily move throughout the world. Compared to transgenic plants and microbes, transgenic insects make the issue of "gene flow" of special relevance. In fact, because many proposed pest management programs rely on the transgenic arthropod population mating with wild populations and persisting in the environment, transgenic arthropods will likely elicit high levels of scrutiny before they can be implemented in pest management programs.

Based on these presentations, the first transgenic insect to undergo risk analysis and implementation in a practical pest management program might involve the release of sterile insects (sterilized in a traditional manner) that contain a transgenic marker gene. Such sterile transgenic insects could not permanently establish in the environment and, as a result, horizontal movement of the marker gene would be unlikely. Even if the marker gene were to move horizontally, the consequences are expected to be minimal. Concerns about releases of transgenic insects that are refractory to disease transmission are expected to be much greater and extensive research is needed to assess the potential consequences of such releases, especially those that would involve permanent establishment in the environment.

What, if anything, is unusual about risk analyses of transgenic insects? The permanent establishment of transgenic insects in the environment, which is key to the success of some proposed pest management programs, means that issues of stability and fitness could be greater with transgenic insects than with transgenic crops, which are protected and cared for by farmers. Furthermore, releases of transgenic insects raise risk issues more like those associated with transgenic fish, because many insects are able to disperse over large distances and could establish permanently in new and undesired environments, causing negative ecosystem effects.